CLEAVAGE IN DIDYMIUM MELANOSPERMUM (PERS) MACBR.

R. A. HARPER

WITH PLATES XI AND XII

Many recent papers have confirmed the contention that cell division in the sporanges of algae and fungi is a process of progressive cleavage by surface furrows as against the older conception of simultaneous division by cell plate formation. Still the unique type of cell division by repulsion of the coarser cell inclusions and their heaping up in neutral planes which delimit the oospheres, described by Farmer and Williams (11) for the oogones of Fucus still remains unquestioned and little has been added to our knowledge of the mechanics of the cleavage process. The older authors were for the most part dominated in their descriptions of spore formation in sporanges, first by the erroneous theory of cell formation put forth by Schleiden, and later by the conceptions of cell plate formation derived from studies on the higher plants. This older literature has been several times adequately summarized and need not be referred to further here (15, 29).

Timberlake (31) describes the swarm spore formation in Hydrodictyon as taking place by means of furrows which cut in from both the plasma membrane and the tonoplastic surface of the primordial utricle. Klebs's (18) figures also certainly suggest that the division is a progressive process.

Klebahn (17) speaks of the division of the oogonia in Sphaeroplea as a cleavage and his figure (no. 2) shows furrows cutting up the multinucleated oogone into the eggs. He does not describe the process of division in the antheridia.

Swingle (29) has proved beyond question that in Rhizopus and in Phycomyces the cell division consists in a progressive cleavage by which the multinucleated spore-plasm is cut up into the definitive spores. In Rhizopus the furrows originate primarily from the periphery; in Phycomyces they originate largely from vacuoles in the spore-plasm. The process in these forms is similar to that in the sporanges of Sporodinia and Pilobolus (16) but with striking and characteristic differences.

Conard (6) finds the process of spore formation in *Lycogala exiguum* entirely similar to that I have described for Fuligo (14). Rytz (28) finds that in *Synchytrium succisae* nuclear division continues during cleavage as I have described for *Synchytrium decipiens* and for *Fuligo varians* and describes the cleavage process as essentially similar to that in *S. decipiens*. Rytz's figures establish the existence of surface furrowing beyond question for this form.

Bally (2) is inclined to accept the conception of cleavage by surface furrows rather than by cell plate formation for *Synchytrium taraxaci*, though stating that his material did not show the stages necessary finally to settle the question. Tobler (33) believes that cleavage takes place by surface furrows in *S. pilificum* and *S. pyriforme*.

A number of other recent authors, supposedly using modern methods of technique, still report cases of so-called simultaneous cell division of multinucleated cell bodies. Baum (5) claims that in Chlamydomucor and Coprinus and a number of other forms simultaneous formation of a cell plate across the entire hypha may occur, while admitting that in hyphae with a central vacuole the cell plate is regularly formed as a peripheral ring which progressively widens inward. He admits further that this same progressive formation of a cell wall may occur in hyphae which are filled with dense cytoplasm through their entire cross section. Baum holds that the essential feature in the process in both cases is the accumulation of granular material to form a cell plate, which is then transformed into a wall and in this he follows the older views of Strasburger. These granules he identifies with the so-called cellulin granules of Pringsheim. Baum's evidence for the existence of simultaneous formation of a cell plate across the entire diameter of a hypha is quite inadequate. His figure 1, *Tafel III*, probably represents a stage after the cell division is complete.

Davis (7) describes the formation of zoospores in *Saprolegnia mixta* as a process of cleavage from the central vacuole outward and accepts the account of earlier authors as to the loss of turgor of the sporange when cleavage is complete and as to the subsequent apparent fusion of the newly delimited spores. His figures 33 and 34 are, however, quite unlike any cleavage process described elsewhere and he asserts that the final step in the separation of the spores consists in the breaking down of fine strands of cytoplasm by which the spores have been connected. For Derbesia, Davis (8) describes and figures the cleavage in the sporange as taking place by branching furrows from the periphery inward.

Löwenthal (20) describes zoospore formation in Zygorhynchus as simply the bounding off of a portion of protoplasm about each nucleus; but his figures show no stages in the process nor evidence as to how it takes place.

Kusano (19) claims to be able to distinguish two types of cell division in *Synchytrium puerariae*. The first type is a progressive cleavage by broad furrows leading to the formation of multinucleated sporanges. In the second type the sporanges are formed "by the precipitation of partitions in the compact cytoplasmic mass." Kusano's figure 6 probably represents young sporanges after cleavage is completed and growth has begun though he argues against this interpretation. It is an old and familiar observation which Wager (34) re-emphasizes that after cleavage is complete the spores may swell and grow so as to press upon each other to the extent of making the lines which mark their boundaries very inconspicuous, especially in living material. It is this condition which has apparently deceived Kusano. Kusano further erroneously identifies his second type of division with that described by Timberlake for Hydrodictyon. As noted above, however, Timberlake describes the cell division in Hydrodictyon as taking place by furrowing and as progressive. For Rhodochytrium, Griggs (13) says, like Kusano, that he observed both progressive cleavage by surface furrows and simultaneous segmentation and adds that the former was infrequent.

Barrett (4) describes the segmentation of the protoplasm in the sporanges of Blastocladia as proceeding from the periphery inward, much as I have described for *Synchytrium decipiens*, but he adds that the lines of division are first recognized as rows of granules which are at first more or less indefinite but which become more and more apparent till they are seen to outline the spores. There can be little doubt, however, as his figures 30 and 31 suggest, that what he describes as rows of granules are in reality cleavage furrows. For the sporanges of Olpidiopsis, Barrett (3) speaks of the division of the protoplasm as involving the formation of spore centers and states that as far as he could determine the fragmentation was simultaneous throughout the sporange much as described by Dangeard for *Synchytrium taraxaci*. He seems, however, in this case not to have really found the cleavage stages; for his figure 39 certainly represents a condition after cleavage is complete, and his figure 38, as he states, represents a stage in which segmentation has not yet begun. It is interesting to note that Bally

(2) is inclined to discard the erroneous description by Dangeard which makes the cleavage in *Synchytrium taraxaci* simultaneous while Barrett holds Dangeard's account is true also for Olpidiopsis.

Wager (34) notes again that the lines of demarcation of the spore-origins, in the cysts of Polyphagus, appear, disappear and reappear again as the older authors have observed in the sporanges of the Saprolegniaceae. He describes spore formation as due to cleavage from the center outward. When the clefts reach the plasma membrane the sporange contracts and perhaps the spores also swell.

Moreau (23) describes the spore formation in *Circinella conica* as beginning with an extreme vacuolization of the protoplasm. The vacuoles become irregular in form and break the spore-plasm up into fragments. The fragments are irregular amoeboid bodies and remain for some time connected by strands which finally break through. Just how this process is related to the cleavage by vacuoles as seen in Pilobolus and Phycomyces is not clear and Moreau's figures give little idea as to just how the vacuolization results in the fragmentation of the protoplasm. Moreau finds the cleavage in Rhizopus and Phycomyces to be essentially as described by Swingle and holds that, as in Circinella, contraction phenomena are an essential phase of the process. In *Mucor spinescens* Moreau finds that vacuolization of the spore-plasm results in the formation of long strands, with the nuclei in a single series. These strands become nodular or catenoid and break up into uninucleated or sometimes several nucleated spores.

For the Conjugatae and Desmids there is general agreement that as in Cladophora the cell divides by a circular cleavage furrow with simultaneous wall formation. Lutman (21) has described the process in detail for Closterium.

The conditions in algae such as Dictyota and Sphacelaria need further study. Swingle (30) and Mottier (24) describe a peculiar sort of plate formation without the presence of a central spindle. Tuttle (32) describes nuclear division in Oedogonium but says nothing definite as to the method of cell division.

McAllister (22) has recently described a cell plate formation in Tetraspora which is quite like that of the higher plants. The interesting possibility is thus suggested that as McAllister argues those algae which are in the line of ascent to the higher plants may have quite a different method of cell division than that found in Cladophora, Hydrodictyon and other well known types. Whether or not this con-

ception is confirmed there can be no question that, in the best known forms of algal and fungous sporanges in which a multinucleated spore-plasm is cut up into one or relatively few nucleated spores, there is no trace of anything like the cell plate formed in the central spindle of the higher plants.

It is evident that there is as yet no final agreement as to the types of cell division characteristic of the different families of plants nor as to the substantial uniformity of the process even within such a genus as Synchytrium or such a family as that of the Mucorineae. Much more careful descriptive work is needed before the limits of variation and the features common to all types of cell division can be determined. The slime moulds are certainly favorable material for such studies and the comparison of cell division in spore sacs which are so similar in externals as are those of Didymium and the Mucorineae gives an excellent opportunity for determining the essential physical and mechanical features of the process independent of resemblances due to close relationship.

The cleavage process has not so far been described for any of the Myxomycetes with simple spore sacs. The sporange of Didymium with its dome-shaped spore mass, large columella cavity and characteristic columnar stalk imitates a mucor closely though the method of its formation is quite different and the parts cannot be considered as homologous. It is questionable whether the term sporange should be used for such diverse types. De Bary (9) introduced the usage substituting the term sporange for the still more objectionable term peridium as used by the older authors on the assumed resemblances of Myxomycetes and Gasteromycetes. It is, however, hardly worth while in the present state of our knowledge to attempt to give any definite morphological significance to such a term used as it is indiscriminately for organs of ferns, algae and fungi without regard to phylogenetic connections.

While the general shape of the dividing mass is strikingly similar in the sporanges of Didymium and the black moulds, in Didymium the spore-plasm at the time of cleavage is already pierced radially at regular intervals by the threads of the capillitium, while in the moulds it is practically a homogeneous mass. It is possible also in Didymium to obtain a view of the whole section of the dividing mass in a fashion not possible in the case of Fuligo and thus to study the mechanical changes in such a multinucleated cell regarded as a unit.

I have photographed the principal stages for the purpose of bringing

out the essential features of the cleavage process free from all possible bias as to the distribution of the nuclei, the angles which the cleavage furrows make with each other, the outlines of the whole mass and the general progress of the process from the periphery to the interior of the spore-plasm. The material was collected near Madison, Wis., fixed in Flemming's weaker solution and stained in most cases with the triple stain though certain of the photographs are from iron haematoxylin preparations.

At a stage when the sporange has reached its full size (fig. 1) and is still milk-white in color the spore-plasm is dense and finely granular in the peripheral region and in the region immediately adjacent to the columella. The middle zone of the sporange on the other hand is still very vacuolar and almost foamy. This represents a condition in which the condensation of the protoplasm in preparation for the formation of spores is complete, except in the interior of the mass. The persistence at this stage of numerous vacuoles in this central region perhaps indicates that a gradual reduction of the cell sap has already been taking place during the early development of the sporange. If the sap passes off by evaporation the vacuoles will persist longer in the central region of the protoplasm.

The capillitium is already formed before the condensation of the protoplasm has been completely accomplished (fig. 2). It consists in Didymium of fine, smooth threads which pass radially outward from the central, dome-shaped columellar cavity to the peridium. The capillitial threads are attached at each of their ends, and we have thus in some respects a condition similar to that in Stemonitis where the capillitium consists of threads branching from a central stalk-like columella and ending peripherally in a very delicate network beneath the peridium. In Stemonitis the radial branches of the capillitium subdivide very freely; in Didymium, on the other hand, the capillitial strands branch scarcely at all. When they do divide the branches form a very acute angle with each other and run on almost parallel toward the periphery. As a result it is not difficult to find in sections lying in the proper plane some fibers that run from the center to the periphery in the plane of the thin microtome section. When mature the capillitial threads are smooth and polished and dark colored. In these early stages the protoplasm is in most intimate contact with the surface of the capillitial threads throughout (figs. 1 and 2). There is no tendency to the formation of vacuoles about the threads nor for

the protoplasm to shrink away from them in fixation. At their inner and outer ends the threads swell slightly and become hollow where they join the peridium and columella respectively. Throughout the rest of their extent they are solid and show no surface sculpturing nor markings of any kind.

At this stage the nuclei are scattered rather uniformly through the protoplasmic mass (figs. 1 and 2). With the magnification used for figure 2 the nuclei appear as hardly more than dots and those less deeply stained are scarcely visible. In some cases there is a tendency for the nuclei to be surrounded with a vacuole. This, however, is true of relatively few of the nuclei in any particular section, while the remainder show themselves in close contact with the cytoplasm over their whole surface. Such cases can hardly be due to shrinkage in fixation, since they are scattered among other nuclei which show no such peculiarity. Nuclei enclosed in vacuoles have been observed in the root hairs of Chara and Debski (10) has shown that in this case the vacuoles do arise during fixation.

The nuclei at this stage are of very unequal size. The smallest nuclei are extremely dense chromatic masses. In some of these it is almost impossible to make out a nucleole; in others, however, and especially in those that are a little larger, by the use of the triple stain a red nucleole can be conspicuously differentiated from blue chromatin as in figure 7. I have not found any nuclear division figures in my sections at this earliest stage, though they are frequent at a very little later stage. We may distinguish three distinct types of nuclei at this stage on the basis of their size and appearance. The type of smallest size is that just described. Those of middle size are abundant and very typically differentiated. The nucleole is sharply defined from the chromatin, is globular in form and dense but transparent and stains bright red. The chromatin consists of strands and granules forming the ordinary netted or reticulated figure of resting nuclei. The nuclear membrane is sharply defined and the nuclear cavity contains a considerable amount of clear cell sap in the meshes of the chromatin network. Nuclei of the third type are distinctly larger, and show very different staining qualities than the smaller nuclei. Their general consistency is very like that of the cytoplasm and they do not appear nearly as conspicuous as do the other two types. Their nuclear membranes are, however, very sharply defined. Their nucleoles also are bright red stained globules no larger than the nucleoles of

the previous type, although the nucleus itself may have a diameter $\frac{1}{3}$ to $\frac{1}{2}$ greater. The chromatin shows very little affinity for ordinary chromatin stains and appears almost homogeneous throughout the nuclear cavity. There is no sharp distinction of chromatic strands and nuclear sap to be made out. The chromatin is apparently distributed in the form of fine granules throughout the nuclear cavity. The condition of these nuclei resembles somewhat that found in the nuclei of the onion root tip at some distance back from the apex where they are not dividing rapidly and pass over into a resting condition.

The homogeneous dense stage in the development of the sporange is followed immediately by a condition which apparently represents a further contraction and loss of cell sap on the part of the protoplasm. At this stage we find abundant vacuoles, or what appear at first glance as vacuoles, arising in the peripheral region of the spore sack (figs. 3, 4, 5). These figures, as is also the case with figures 2, 6–10, 15 and 17, represent sections cut more or less horizontally through the developing spore sack, the columella cavity appearing as a more or less circular opening in the center. A little closer examination shows that each of these apparent vacuoles is pierced by a capillitial thread which runs straight through its central axis or may be slightly curved (figs. 9, 15, 17). These apparent vacuoles are in reality cavities formed by the withdrawal of the protoplasm from the surface of the capillitial threads at particular points. It is plain that the protoplasm is bounded off against the surface of the capillitial thread by an osmotic membrane, and that it has exuded liquid through this membrane into a space formed about the surface of the thread. The increase in amount of this exuded liquid leads to the formation of an almost globular droplet, which on superficial observation has all the appearance of an intra-cytoplasmic vacuole. Several of these droplets may be formed in a series not very far apart on the same capillitial thread. The row of watery droplets is in a sense suspended on the capillitial thread. It is to be seen at once that these droplets are in the strictest sense exterior to the protoplasmic mass, since the membrane of the apparent vacuole which encloses them is in reality continuous along the capillitial thread with the external plasma membrane of the whole sporange. The droplets are not of course perfectly spherical, but form more or less extensive catenoid-like series on the capillitial threads. Their formation seems to consist, as noted, in a further extrusion of what we may call cell sap from the protoplasm, the exudation now taking

place along the surface of the capillitial threads as well as upon the external surface of the protoplasmic mass as a whole. These sap cavities on the capillitial threads become very abundant, extending throughout the whole peripheral region of the spore-plasm. They next become connected along the threads and we have thus as it were canals running radially in the peripheral region of the cytoplasm and filled with a watery extruded liquid. Figure 5 shows the apparent vacuoles somewhat ellipsoidal in form as they run together. This change in their form and their resulting confluence are no doubt due to the continued extrusion of water from the protoplasm in preparation for spore formation. These watery sheaths open to the exterior at the ends of the threads, forming, as noted, tubular canals about the capillitial threads leading down into the protoplasmic mass. The extruded cell sap now apparently flows out quite freely, leaving the spore-plasm once more quite dense, but it does not again come into close connection with the surfaces of the capillitial threads (figs. 6 and 8). Such radial sections at this stage show thus what appear to be clefts running through the spore-plasm. That they are not furrows but are merely tubular depressions enclosing the capillitial threads is clearly shown in tangential sections of this same stage (fig. 10).

The contraction of the protoplasm and extrusion of liquid occurs also in the region next the columella so that we have the same appearance of clefts here (figs. 6, 8), and as the contraction continues it is possible to trace these tubular openings about the capillitial threads from the periphery to the center as continuous openings through the protoplasm (fig. 9). The appearance of these radial clefts sharply differentiates this stage from the earlier conditions shown in figure 2. As noted, a tangential section at this stage (fig. 10) shows the protoplasm pierced by rounded or angular openings in each of which the cross section of a capillitial thread is found. The angular openings represent the later stage and are formed from the rounded openings by the formation of cleavage furrows from their surface. These furrows cut into the spore-plasm and tend to divide it up into radially placed prisms or cylinders. Sometimes more than one capillitial thread will be found in a single opening. Very soon the furrows from adjacent openings come together making irregular branching series of clefts. More or less horizontal radial sections at the stage when the furrows first begin to form about the capillitial cavities are shown in figures 9 and 11. We see in such sections that the end of each prism

or cylinder of spore-plasm tends to be rounded off so that the clefts are wider at the surface than they are deeper down in the sporange.

The furrows which have originated from the capillitial openings curve and branch in the peripheral region in such a fashion that they come to lie in a plane more or less tangential to the surface of the sporange and in this way begin to cut off blocks from the ends of the cylinders of spore-plasm formed in the initial cleavage stages. These tangential furrows begin to be formed before the radially placed cylinders and prisms have been entirely separated from each other. The cleavage is in reality a progressive process working from the surface of the spore sack inward, both by radially and by more or less tangentially placed furrows. An early appearance of these tangential furrows is shown in figure 9, and a slightly later stage in which the first peripheral blocks are almost or entirely cut off is shown in figure 11 at *a* and *b*.

These same stages in the cleavage process can be observed also in the inner layer of the spore-plasm next to the columella cavity (fig. 11). But in this inner layer the cleavage is always a little less advanced than in the outer layer. The whole process parallels apparently a continuation of the extrusion of cell sap which began at an earlier stage in the formation of the sap cavities around the capillitial threads (figs. 3 and 4), and the extrusion of sap is apparently most rapid where evaporation can go on most rapidly. We cannot, of course, regard the process of cutting up the spore-plasm as a mere matter of the drying out of the protoplasm due to evaporation. The clefts and furrows may be more or less filled with liquid at all times, and there is thus only a minor difference possible between the superficial and the central portions of the spore-plasm. Still, loss of water by way of the columella and stipe, which is filled with coarse concretions (see fig. 18), is doubtless slower than from the outer surface of the sporange and we find that cleavage is also slower next the columella than on the peripheral surface of the spore-plasm. There seems to be an obvious parallel between facility for water loss and rate of cleavage, but this in no wise excludes the possibility that chemical changes in the plasma membrane also favor the active extrusion of moisture through all these cleavage stages.

At a stage when the shrinkage about the capillitial threads has led to the appearance of the radial clefts shown in figures 6–10, the nuclei show quite commonly a tendency to be arranged in series

adjacent to the shrinking protoplasmic surfaces (fig. 7). In sections this arrangement of the nuclei leads to their apparent distribution in rows along the radial clefts. This appearance in section, of course, is due to the fact that the nuclei really tend to occupy a layer of the cytoplasm next adjacent to the plasma membrane bounding the capillitial cavities. The majority of the nuclei appear in such rows, although a considerable percentage are irregularly distributed. In radial sections a row of the sort described is frequently to be seen in the protoplasm with no apparent capillitial opening near it. An examination of the next adjacent sections, however, will always show that these rows are either immediately above or below a capillitial thread.

The interesting fact is to be further noted that at the stage when the nuclei tend to show this characteristic arrangement along the cleavage surfaces a very high percentage of them are in the equatorial plate stage of division. This is especially true of the nuclei that have this characteristic position. Probably 75 per cent. of the nuclei at this stage that are arranged along the cleavage surfaces are in this stage of division. In figure 7 two of the nuclei in the equatorial plate stage are shown in polar view. The three resting nuclei may be distinguished by the presence of their nucleoles. Deeper in the protoplasm the nuclei may or may not be dividing. The further fact may be noted that the long axis of the spindle is quite commonly more or less parallel to the surface of the protoplasmic mass adjacent to which it lies. This is true only of the nuclei lying near the plasma membrane; those deeper in the protoplasm show no such orientation of their spindles.

It is interesting to note that a very considerable portion of the nuclei in Didymium at this stage are in a resting condition and that these resting nuclei are scattered irregularly amongst those which are dividing. The conditions in this respect are in sharp contrast with those in Fuligo where, during the cleavage stages, in any particular region of the aethalial mass, all the nuclei will be found in division at one stage or another. It is also in contrast with the conditions in Enteridium and Lycogala in which practically all the nuclei in a particular spore sack divide simultaneously. Series of spore sacks adjacent to each other may be found in which every nucleus is in the equatorial plate stage of division.

In Didymium it seems doubtful whether all the nuclei undergo division during the cleavage stages, though it is of course possible that the nuclei which are found in the resting condition at any par-

ticular stage may divide at a little later period. Since, however, as indicated above, the process of cleavage is rather rapid, it is quite possible in Didymium that only one nuclear division occurs between the time when the sporanges have reached their full size and the stage of complete ripening of the spores.

As noted, the cleavage process is initiated both from the outer and inner surfaces of the spore-plasm. A stage when almost an entire series of peripheral blocks have been cut off is shown in figure 12. At this stage the cleavage next to the columella is markedly less advanced. In the segments which have been cut off the nuclei are found to be irregularly distributed in their interior. There is apparently no tendency now for the nuclei to take a position just next to the plasma membrane. In figure 15 a later stage in which cleavage has gone still farther is shown. Cleavage is advancing also in the region of the columella but is markedly behind that on the periphery. Certain of the outer blocks have begun to subdivide by surface furrows at this stage. The planes of the cleavage furrows become very irregular as the process advances and the clefts which marked the course of the capillitial threads gradually disappear.

Tangential sections of these stages show the formation of the cleavage furrows very clearly (figs. 13 and 14). In the periphery of such sections the irregular blocks of protoplasm seem entirely free from each other, a little nearer the center the furrows are seen advancing and branching to cut off blocks of all sizes. Still deeper and at the center we find cross sections of rounded and angular cavities, which indicate the first stages in the formation of the cleavage furrows from the surfaces of the capillitial openings. The pushing out of the first clefts from these tubular capillitial cavities is very well shown in these figures. The cleavage is seen to be strictly progressive from the surface inward, and further the surfaces adjacent to the capillitial cavities are in their relation to cleavage exactly equivalent to the external surfaces of the mass of spore-plasm as a whole. The plasma membranes of the capillitial openings are the source of cleavage furrows to even a greater degree than the original surface plasma membrane of the spore sack as a whole. Figure 16 shows a drawing of a multinucleated block of the spore-plasm as it is being cut up into smaller fragments. It is plain here, as in the photographs, that the furrows cut into entirely undifferentiated protoplasm with no hyaline zones or rows of granules to indicate the direction they will take. Even in

the last stages of cleavage in Didymium I have observed no such condensation of the spore plasm around the nuclei as one finds in Fuligo. The cleavage progresses in the same fashion until we have the condition shown in figure 17, in which both the outer surface of the spore mass and the inner surface have been cut up into small irregular protoplasmic blocks, while the middle region of the spore mass still shows large angular blocks of protoplasm which are being rapidly cut into by furrows over their entire surface. The ultimate result of this progressive furrowing is the formation of uninucleated rounded spores which are of fairly constant size, as shown in figure 18. They lie packed between the capillitial threads which still hold their radial direction running from the central columella to the peridium. The capillitial threads at this stage are so brittle that they break up to a considerable extent in sectioning.

As described, the segments cut off by the cleavage furrows in the earlier stages, here as in Synchytrium, Pilobolus, Fuligo, and many other forms, are multinucleated and of very varying form and size. As the cleavage progresses, however, it is apparent that the distribution of the nuclei, though irregular and superficially considered accidental, is none the less in so far definite that the cleavage furrows never cut off non-nucleated segments and in the end each spore contains a single nucleus. The final cleavage stages here, as I have described in detail for Fuligo, always cut two-nucleated blocks into one-nucleated spores. These are the definitive spores of the slime mould. They show no subsequent growth or nuclear division in the process of ripening.

The appearance of the cleavage figures suggests most strikingly the contraction which accompanies the process. In the period of most active segmentation the blocks of protoplasm lie quite separate from each other. Part of the open space as shown in the figures is doubtless due to shrinkage in fixation and in particular regions some segments have dropped out of the section. Still there can be no question that the free space between the blocks is to a considerable degree the result of the same extrusion of cell sap which led to the opening up of the broad surface furrows in earlier stages.

Rothert (27) long ago observed the escape of cell sap and accompanying shrinkage during spore formation in Saprolegnia. The conditions in Didymium with its radially placed capillitial threads on which in the early stages the extruded liquid accumulates in bead-like

droplets resembling vacuoles are especially favorable for the demonstration of the parallelism between the exudation of water and the formation of the cleavage furrows. I have pointed out the evidence for a similar parallelism in the case of *Synchytrium decipiens*, where the extruded liquid contains substances which are blackened by osmic acid. Kusano (19) has observed the same extrusion of liquid in *S. puerariae* though denying that it is of universal occurrence in this species. The formation of vacuoles from which cleavage furrows may arise in Pilobolus and Phycomyces is undoubtedly a similar phenomenon bearing the same general relation to the cleavage process. Such loss of water from sporanges has long been associated with the ripening processes by which the watery protoplasm of the hyphae or plasmodium is transformed into the condensed and dust-like mass of spores. I have already pointed out the possibility that this exudation of water may at least be a factor in the process of cleavage on the assumption of an analogy between the furrowing of the spore-plasm and the cracking of a drying colloidal mass.

In a recent paper with Dodge (15) we have also pointed out the relation of this same extrusion of water into vacuoles in the early stages of the formation of the sporanges of Trichia to the formation of the capillitium. An essential and doubtless primitive feature of the transition from the expanded vegetative condition to that of the dust-like spores for reproduction in these slime moulds is the exudation of water and it is quite to be expected that from the physical chemical standpoint the formation of the capillitium, the production of thick spore walls and condensed reserve products, and even the process of cell division itself would all develop in a manner most intimately related with and dependent on the fundamental process of getting rid of superfluous moisture. Still as I have elsewhere emphasized cell division in these sporanges cannot be regarded as simply a matter of the drying out and cracking to pieces of a colloidal mass. The extrusion of water into internal vacuoles shows that the process is initiated and controlled by changes in the spore-plasm itself. The fact that the final furrows always separate uninucleated spores is also proof of organization in the segmenting mass.

Visible evidence of this organization such as we find in connection with the processes of cell division in many plant cells is entirely lacking in these sporanges. There seems to be no evidence whatever for the existence of such systems of specially oriented fibrils as are

seen in the central spindle and polar asters and which play so important a rôle in the phenomena of cell division and free cell formation in the higher plants and in the ascus.

None of the mechanical theories of cell division like those of Heidenhain and Kostanecki, which also involve the existence of a special system of organic rays for each nuclear center, can have any application, as I have pointed out before, in cases of progressive cleavage of multinucleated masses such as we have in the spore sacks of the slime moulds and fungi.

Swingle (29) has clearly diagrammed the tension relations involved in the cleavage processes of these sporanges, whether or not the furrowing is really due to localized contraction of the cytoplasm, as he supposes. The cleavage furrows whether originating from the vacuoles, the periphery of the mass or the capillitial canals constitute a complex system of catenoidal surfaces which tend to divide the mass into spherical fragments. Such cleavage surfaces may equally well be conceived as due to exudation of water with increase of surface tension between the cytoplasm and the extruded moisture due to the ripening changes going on in the former, involving, as they probably do, condensation processes with change from highly hydrated proteids to lipoid or other fatty storage products. Such chemical changes may well play a part in the liberation of the energy necessary for the accomplishment of these profound form changes.

I have elsewhere suggested (14, 16) that in the gradual shrinkage and condensation of the spore-plasm the loss of water might be least in the neighborhood of the nuclei and that thus a determining factor in the orientation of the cleavage surfaces would be introduced. The well-known facts as to the effect of acids and alkalies on the imbibition of water by colloids utilized by Pauli (25) in his surface tension theory of the contraction of striated muscle fibers and by Fischer (12), in his theory of oedema may have significance in connection with those processes which involve the exudation of water in the sporange. A localized production of acid in the colloidal spore-plasm involving differentiated capacity for the retention of water might determine the direction of the cleavage furrows. The cleavage planes would naturally follow zones of greatest water loss thus isolating such acid-containing areas. If the nuclei either by reason of their characteristic chemical content (nucleo-proteids, nucleic acid) or by the products of their metabolism could thus become centers of moisture

retention we should have a factor which would tend to such an orientation of the cleavage planes as in the end would produce uninucleated spore units. The chemical changes in the colloids in such acid regions might lead even to the visible differentiation of the hyaline zones which in the later stages of cleavage in Fuligo and the spore embryos of Pilobolus seem to predetermine the planes of the cleavage furrows.

Hofmeister has emphasized the possibility of widely different chemical processes and conditions coexisting in a polyphase colloidal system like the cell because of the low mutual diffusion rates of its various elements and the possibility of such differences in reaction between the nuclear region and the cytoplasmic region of the cell must be recognized. If we add to this conception of the nucleus as a center of water retention the further conception, suggested by its relation to cell plate formation in the higher plants and the ascus, that it is a center for the production of plasma membrane materials, we have two factors which would work in harmony to bring about the process of progressive cleavage as described, since the diffusion outward from the nucleus of substances to be used in forming the plasma membrane would again tend to cause the cleavage planes to pass midway between any given pair of nuclei.

INDEX TO LITERATURE

1. **Bally, W.** Die Chytridineen im Lichte der neueren Kernforschung Mycolog. Centralbl. **2**: 289. 1913.
2. **Bally, W.** Cytologische Studien an Chytridineen. Jahrb. wiss. Bot. **50**: 95. 1911.
3. **Barrett, J. T.** Development and sexuality in some species of Olpidiopsis (Cornu). Fischer. Ann. Bot. **26**: 209. 1912.
4. **Barrett, J. T.** The development of Blastocladia strangulata, n. sp. Bot. Gaz. **54**: 353. 1912.
5. **Baum, Joh. Pet.** Zelltheilung in Pilzhyphen. Diss. Fulda. 1900.
6. **Conard, H. S.** Spore formation in Lycogala exiguum Morg. Iowa Acad. Sci. **17**: 83. 1910.
7. **Davis, B. M.** Oogenesis in Saprolegnia. Bot. Gaz. **35**: 233. 1903.
8. **Davis, B. M.** Spore formation in Derbesia. Ann. Bot. **22**: 1. 1908.
9. **De Bary, A.** Morph. u. Physiol. der Pilze, Flechten u. Myxomyceten. 1866.
10. **Debski, B.** Beobachtungen über Kernteilung bei Chara fragilis. Jahrb. wiss. Bot. **30**: 227.
11. **Farmer, J. B. and Williams, J. L.** Contributions to our knowledge of the Fucaceae, their life-history and cytology. Trans. Roy. Soc. Lond. Ser. B. **190**: 623. 1898.

12. **Fischer, M. H.** Oedema. New York, 1910.
13. **Griggs, R. F.** The development and cytology of Rhodochytrium. Bot. Gaz. **53**: 127. 1912.
14. **Harper, R. A.** Cell and nuclear division in Fuligo varians. Bot. Gaz. **30**: 217. 1900.
15. **Harper, R. A., and Dodge, B. O.** The Formation of the Capillitium in certain Myxomycetes. Ann. Bot. **28**: 1. 1914.
16. **Harper, R. A.** Cell Division in Sporangia and Asci. Ann. Bot. **13**: 467. 1899.
17. **Klebahn, H.** Die Befruchtung von Sphaeroplea annulina Ag. Schwendener Festschrift, p. 81.
18. **Klebs, G.** Ueber die Bildung der Fortpflanzungs-zellen bei Hydrodictyon utriculatum, Roth. Bot. Zeit. 1891.
19. **Kusano, S.** A contribution to the cytology of Synchytrium and its hosts. Bull. College of Agric. Imp. Univ. Tokyo, **8**: No. 2. 1909.
20. **Loewenthal, W.** Weitere Untersuchungen an Chytridiaceen. Archiv Protistenkunde, **5**: 221. 1905.
21. **Lutman, B. F.** Cell and nuclear division in Closterium. Bot. Gaz. **51**: 401. 1911.
22. **McAllister, F.** Nuclear division in Tetraspora lubrica. Ann. Bot. **27**: 681. 1913.
23. **Moreau, F.** Recherches sur la reproduction des mucorinées et quelques autres thallophytes. Thèse de l'Université de Paris, 1913.
24. **Mottier, D. M.** Nuclear and cell division in Dictyota dichotoma. Ann. Bot. **14**: 163. 1910.
25. **Pauli, W.** Ergebnisse der Physiologie. **6**: 126. 1907; and Kolloidchemie der Muskelkontraction. Dresden, 1912.
26. **Pfeffer, W.** Zur Kenntniss d. Plasmahant u. die Vacuolen. 1890.
27. **Rothert.** Die Entwicklung der Sporangien bei den Saprolegnieen. Beitr. zur Biol. der Pflanzen, **5**: 291. 1892.
28. **Rytz, W.** Beiträge zur Kenntniss der Gattung Synchytrium. Centralbl. f. Bakt. 2 Abt. **18**: 635 u. 799. 1907.
29. **Swingle, D. B.** Formation of the spores in the sporangia of Rhizopus nigricans and of Phycomyces nitens. U. S. Dept. Agric. Bur. Plant Ind. Bull. 37. 1903.
30. **Swingle, W. T.** Zur Kenntniss der Kern- und Zelltheilung bei den Sphacelariaceen. Jahrb. wiss. Bot. **30**: 297. 1897.
31. **Timberlake, H. G.** Development and structure of the swarmspores of Hydrodictyon. Trans. Wis. Acad. Sci. **13**: 486. 1902.
32. **Tuttle, A. H.** Mitosis in Oedogonium. Journ. Exper. Zool. **9**: 143. 1910.
33. **Tobler, G.** Die Synchytrieen. Archiv. Protistenkunde **28**: 141. 1913.
34. **Wager, H.** The Life-history and cytology of Polyphagus Euglenae. Ann. Bot. **27**: 173. 1913.

EXPLANATION OF THE PLATES XI AND XII

Figures 7 and 16 were drawn with the aid of the camera lucida from preparations stained with the triple stain. Zeiss apochromatic objective 3 mm. N.Ap. 1.3, ocular 8.

The microphotographs were made with the Zeiss apochromatic objective 16 mm. Oc. 4 with Cramer's micro-ray filter No. 7 except in case of the iron haematoxylin preparations, figures 15, 17, and 18.

PLATE XI

FIG. 1. Part of radial section of young sporange before cleavage, showing two capillitial threads, the vacuolated condition of the middle zone of the spore-plasm and the distribution of the nuclei.

FIG. 2. Entire horizontal radial section of sporange at stage when the spore-plasm has become quite homogeneous, showing radial capillitial threads and the distribution of the nuclei. Columella containing reticulated gelatinous material extruded in formation of the sporange. × 90.

FIG. 3. Horizontal section, later stage, showing rows of extruded droplets of cell sap on the capillitial threads. Columella showing lobed concretion. × 90.

FIG. 4. Like fig. 3. Well-developed concretion in columella. × 90.

FIG. 5. The droplets of cell sap have become ellipsoidal preparatory to flowing together. × 90.

FIG. 6. Horizontal section later stage with thin, watery sheaths around the capillitial threads. × 90.

FIG. 7. Drawing showing arrangement of the nuclei along the plasma membrane of the watery sheath surrounding a capillitial thread. × 800.

FIG. 8. Horizontal section showing a little later stage than fig. 6. × 90.

FIG. 9. Horizontal section showing the watery sheaths around the capillitial threads more strongly developed. Cleavage beginning at (*a*). The checking of the protoplasm is artefact.

PLATE XII

FIG. 10. Tangential section showing cleavage furrows forming about the capillitial openings. × 90.

FIG. 11. Stage a little later than shown in fig. 9. Cleavage at (*a*) and (*b*). × 80.

FIG. 12. Vertical section showing early stage in cleavage. × 90.

FIG. 13. Tangential section showing cleavage. × 90.

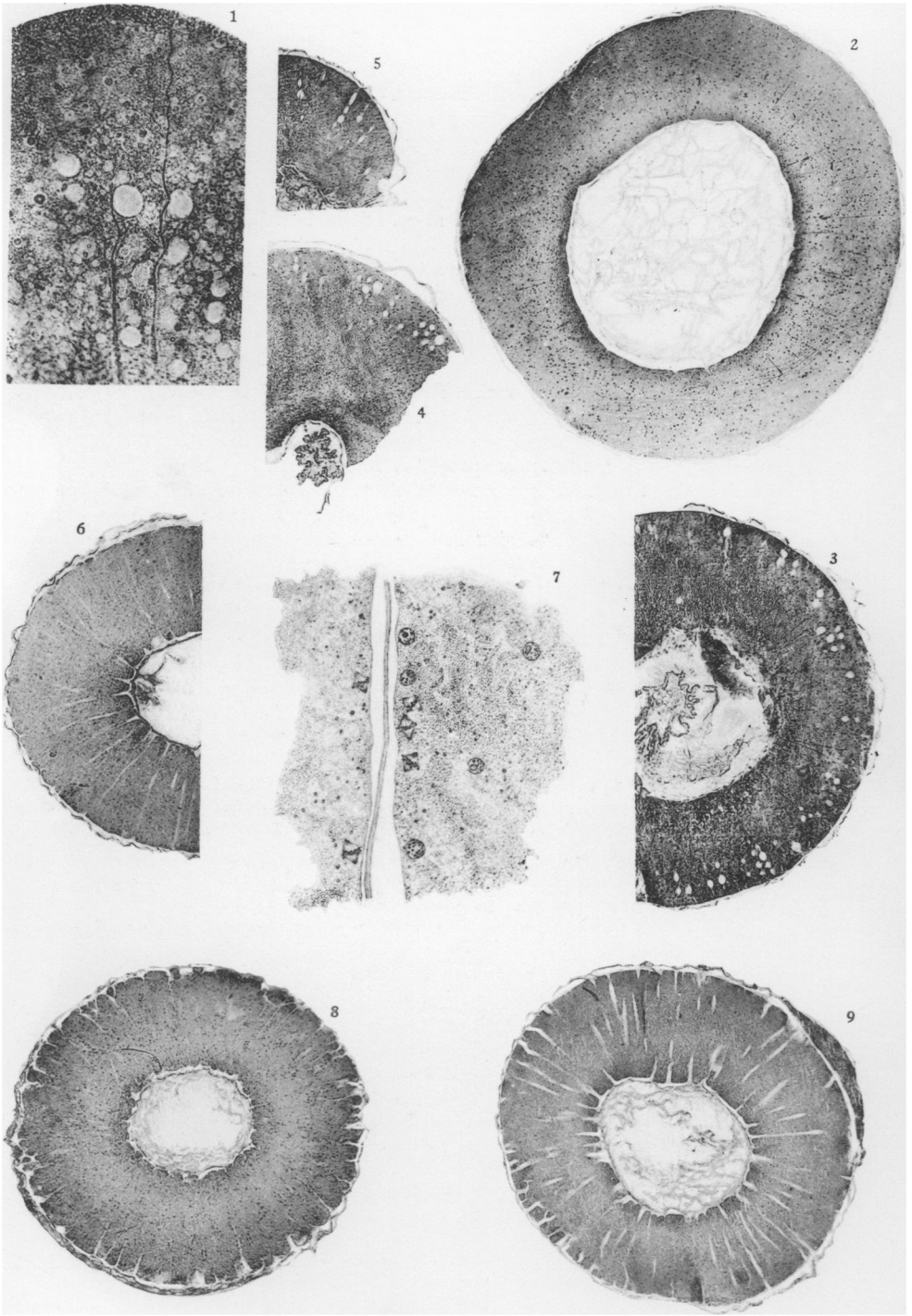
FIG. 14. Tangential section showing a little later stage than fig. 13. × 90.

FIG. 15. Advanced stage in cleavage. × 90.

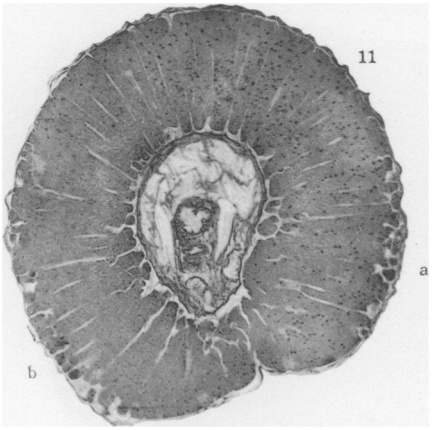
FIG. 16. Drawing of segmenting block of spore-plasm showing cleavage furrows and resting nuclei, others in equatorial plate stage. × 650.

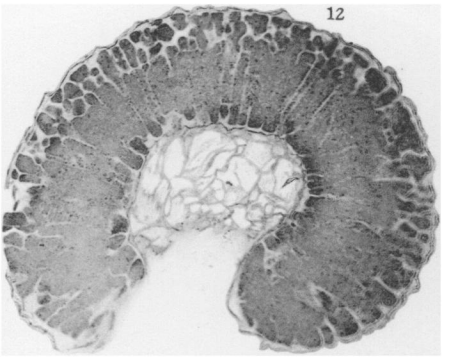
FIG. 17. Late stage of cleavage. × 65.

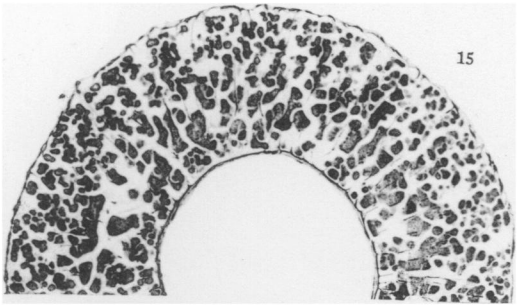
FIG. 18. Vertical section of entire sporange showing spores, stipe and hypothallus. Stipe and hypothallus filled with granular concretionary material.

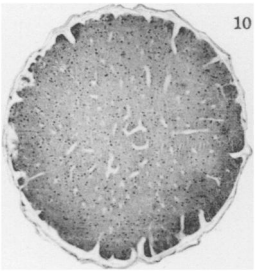


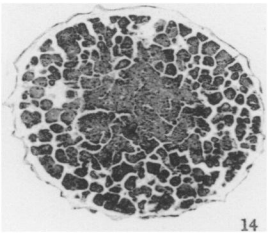
HARPER: CLEAVAGE IN DIDYMIUM.

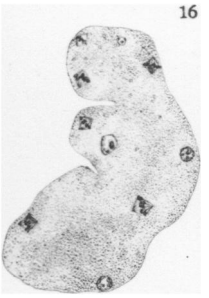


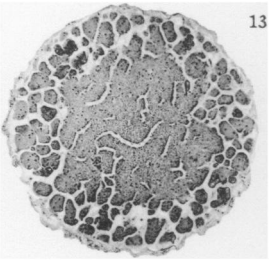


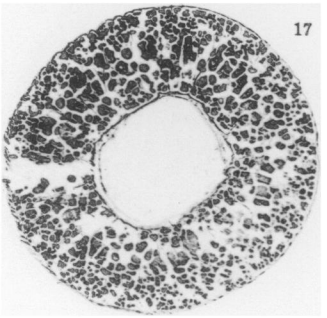


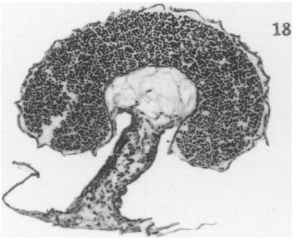












HARPER: CLEAVAGE IN DIDYMIUM.